

Azadirachtin based Pesticide Neem-On Induced Histopathological Alterations in kidney of *Labeo rohita*



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Abstract : The present study attempts to investigate the histopathological effects of Neem (*Azadirachtin*) on the histo-pathological alterations in kidney of *Labeo rohita*. The *Labeo rohita* was treated for acute and chronic exposures. The acute exposure was given for four days while the chronic exposure was given for 30 days. The two sub lethal concentrations of 2.844 ppm and 4.266 ppm (1/10 and 1/15 of LC₅₀) were selected. After the exposure of two sublethal doses for 4 days fishes showed cloudy swelling, dilation of lumen, tubular vacuolation, appearance of pyknotic nuclei, necrosis and shrinkage of lumen in the kidney. However, after 30 days of exposure to both the sublethal concentrations, the kidney showed clearly shrinkage and fragmentation in glomeruli situated inside Bowman's capsule. Significant damages were seen in the hematopoietic tissue.

Keywords: Neem (*Azadirachtin*), *Labeo rohita*, Necrosis in glomerulus of Bowmans capsule.

Introduction

It is unanimously accepted that the pesticides contaminate the aquatic environment and ecosystem and ultimately causes damage to the organs of aquatic animals in general and fishes in particulars. Histopathological alterations have been widely used as biomarkers for health evaluation of organism exposed to pollutants and can be used as warning symptoms for organism health. Namdeo and Tembhe (2017) reported that gills of *Catla catla* show anticholinergic effect and histological alterations due to the treatment of Chlorpyrifos and *Datura stramonium* leaf extract. Roy and Bhattacharya (2006) studied the effect of arsenic on the kidney of *Channa punctatus* and reported arsenic induced histopathology and causes of synthesis of stress proteins in liver. Camargo and Martinez (2007) reported the histopathology of gills, kidney and liver and digestive tract in Neotropical fish caged in an urban stream. Tilak *et al.* (2001) found that increased uses of pesticides in agriculture have introduced serious hazards to the animal beings including fishes. Prolonged exposure to some of these chemicals causes disturbance in the physiological activities beside other pathological features. Kidney is the major detoxification organ for the removal of xenobiotics for long term as well as acute exposure (Prashanth, 2011).

In spite of xenobiotics, extracts of many plants showed pathological activities the organs of fishes (Singh *et al.*, 2007). Neem (*Azadirachta indica* A. Juss) is a traditionally highly esteemed medicinal tree easily available in the Indian subcontinent. The neem tree, *Azadirachta indica* is so far the most promising example of the plant currently being used. The principal active ingredient of neem is tetranortriterpenoids (limonoids) used for control of pests and also other harmful animals tree (Kreutzweiser, 1997;

Mondal *et al.*, 2007; Morgan, 2009; Okumu *et al.*, 2007; Punzo and Parker, 2005; Shafeek *et al.*, 2004; Shanmugasundaram *et al.*, 2008; Winkaler *et al.*, 2007). Neem extract is considered to be of low toxicity to non-target organisms; however, water extracts from various parts of the Neem tree have been reported to cause respiratory problems and delayed growth in fishes and also to interfere with homeostasis thus affecting performance (Omeregic and Okpanachi, 1997). Deshmukh and Pariyal (1992) have also reported the toxic effects of Neemark on *Tilapia mossambicus* (Peters).

The influence of neem on Indian major carp, *Labeo rohita* is very limited, although it is important Indian major carp, widely cultivated in India and extensively edible. It is therefore, thought to study the effect of Neem on the histopathology of the kidney of *Labeo rohita*.

Materials and methods

Pesticide

Neem-On manufactured by Jai Kissan Agro Pvt. Ltd., Sangam Nagar, Indore, (M.P.) India, was purchased from the local market and was used for evaluation of the toxicity to the fish.

Live specimens of healthy and active adult *Labeo rohita* (weighed 55±3gm and length 14cm±1) were obtained from Patra and Bhadbhada fish farms, Bhopal (M.P). They were brought to laboratory carefully in oxygen filled polythene bags. They were disinfected by giving a bath for five minutes in week KMnO₄ solution before they were transferred to glass aquaria filled with dechlorinated water. The fishes were acclimated to the laboratory conditions for at least 20 days prior to the experiment. During acclimatization fishes were fed daily in morning with

commercial fish feed. Water was replaced every 24h after feeding in order to maintain a healthy environment for the fish during acclimation and experimental period. This ensures sufficient oxygen supply for the fish and the environment is devoid of any accumulated metabolic wastes. Dead fishes were removed immediately to avoid fouling of the water. Dissolved oxygen was maintained using aerator. Physico-chemical property of water was checked throughout the experiments according to APHA/AWWA/WEF (2005). The feeding was stopped before 24 hrs prior to end of exposure periods.

Treatment protocol

Fishes were divided into five groups of ten each. Group-I : served as control, in this group no toxicant was used; Group II: the fishes were given 4.266 ppm as sub-lethal concentrations ($1/10^{\text{th}}$ of LC_{50}) of Neem-On daily for 96 hrs. Group-III: fishes were given 2.844 ppm as sub-lethal concentrations ($1/15^{\text{th}}$ of LC_{50}) of Neem daily for 96 hrs.. Group-IV, the fishes were subjected to the treatment of 4.266 ppm sub-lethal concentrations ($1/10^{\text{th}}$ of LC_{50}) of Neem daily for 30 days. Group V: fishes were given 2.844 ppm sublethal concentrations ($1/15^{\text{th}}$ of LC_{50}) of Neem-On daily for 30 days.

Histopathological studies

At the end of experiments, five fishes were removed from each group to study the effect of toxicant on the histology of kidney. The fishes were dissected and kidneys were removed immediately and fixed in Bouin's fluid for 24hrs. and processed according to standard procedure of routine micro technique. The blocks were prepared in paraffin wax and sections were cut on rotatory microtome to a thickness of 6 to 8 μ . For staining the double staining method was followed by using Haematoxyline and Eosin as a stains and mounting was done in DPX. The histological anomalies were studied in slides under microscope and desired areas were photographed at 400X.

Results

Histology of kidney of control fish

The basic unit of kidney in fish consists of a renal corpuscle, Bowman's capsule and glomerulus. The proximal tubule, intermediate segment, distal tubule and collecting duct of renal tubules, were dissected. Proximal tubules have prominent brush borders (Microvilli) bathed in the vascular bed in the interstitial tissues. Distal tubules and collecting ducts, both devoid of brush borders and are sparsely distributed. The intermediate segments between proximal and distal tubules are rarely seen. The renal corpuscles are located in close vicinity of renal tubules and blood vessels in the interstitial tissue. Pigments and leucocytes are very common in the interstitial tissue (Fig.1).

Histological changes in the kidney of treated fish

96 hours exposure of Neem-on

After the exposure of Neem-on at the sub lethal concentrations 4.266ppm and 2.844ppm of 96hrs fishes showed cloudy swelling, dilation of lumen, tubular vacuolation, appearance of pyknotic nuclei, necrosis and shrinkage of lumen were seen (Fig. 2, 3, 4 & 5).

Chronic exposure 30 days of Neem-on

After 30 days of exposure to sub lethal concentration 4.266 ppm of Neem-on, histopathological examination of the kidney of *Labeo rohita* clearly shows shrinkage of glomeruli, expansion of space inside Bowman's capsule, fragmentation of glomerulus and cloudy swelling. Also loosening and damaging of hematopoietic tissue, tubular vacuolation, necrosis, appearance of pyknotic nuclei, shrinkage and dilation of lumen was evident in Neem-on treated kidney. However, fish exposed to sub-lethal concentration at 2.844 ppm of Neem-on for 30 days showed pathological changes. The most common symptoms of toxic exposure were mild cloudy swelling, narrowing of lumen and appearance of pyknotic nuclei (Fig. 6, 7, 8 & 9).

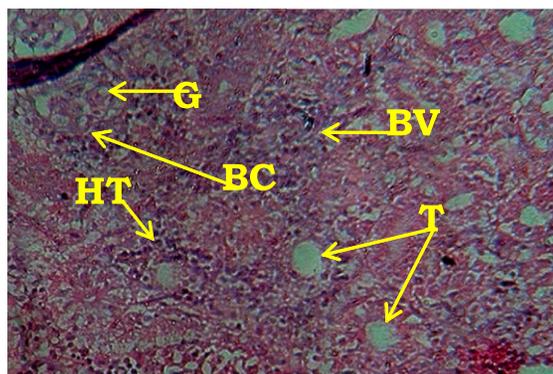


Fig.-1. Photomicrograph of kidney (x400) showing kidney in the control fish.

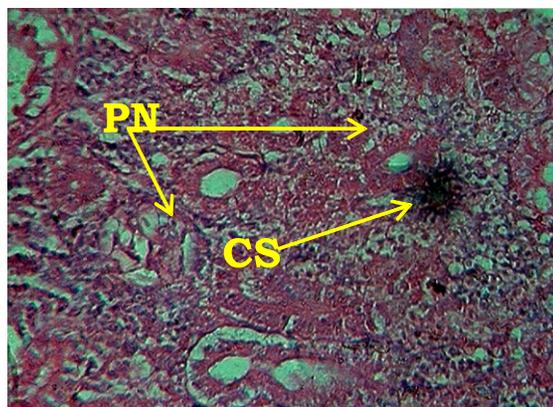


Fig.- 2. Photomicrograph of kidney (exposed to 4.266ppm Neem-On 4 days) showing Glomerulus (G), blood vessel (BV), hematopoietic tissue (HT), kidney tubules (T), pyknotic nuclei (PN), Bowman's capsule (BC). Showing cloudy swelling (CS) and Tissue (HT).

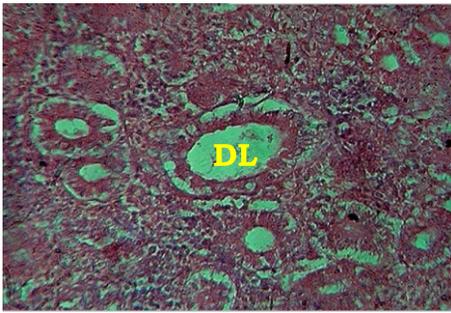


Fig.-3. Photomicrograph of kidney (x400)×400) exposed to 2.844 ppm Neem-On for 4 days showing dilation of lumen (DL).

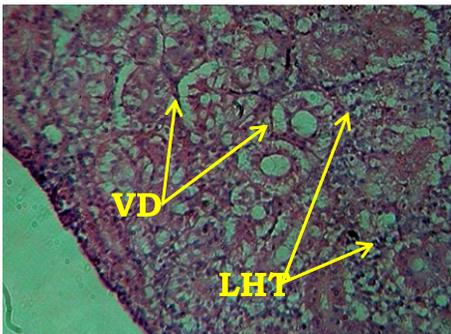


Fig.-4. Photomicrograph of kidney (x400)× exposed to 4.266 ppm Neem-On for 4 days showing vacuolar degeneration (VD) and loosening of hematopoietic tissue (LHT).



Fig.-5. Photomicrograph of kidney (x400)×400) exposed to 2.844 ppm Neem-On for 4 days showing shrinkage of lumen (SL).

Fig.- 9. Photomicrograph of kidney exposed to (x400) 2.844 ppm Neem-On for 30 days showing mild cloudy swelling (CS), narrowing of lumen (NL) and pyknotic nuclei (PN).

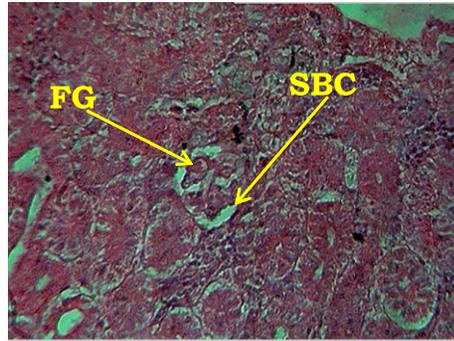


Fig.-6. Photomicrograph of kidney (x400)×400) exposed to 4.266 ppm Neem-On for 30 days showing fragmentation of glomerulus (FG) and expansion of space inside bowman's Capsule (SBC).

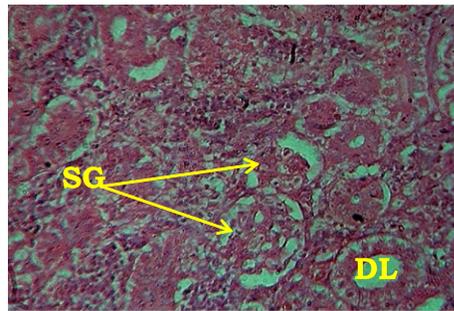


Fig.-7. Photomicrograph of kidney (x 400) exposed to 4.266 ppm Neem-On 30 days showing shrinkage of glomerulus (SG) and dilation of lumen (DL).

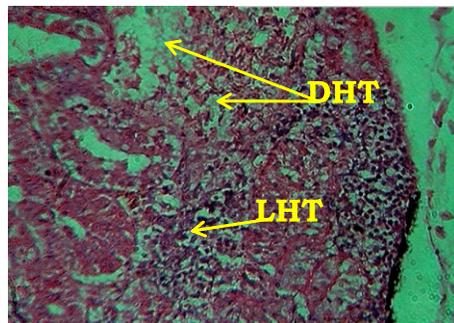
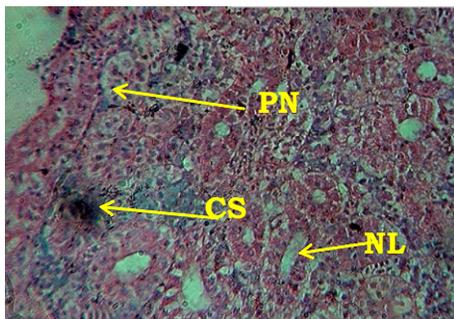


Fig.- 8. Photomicrograph of kidney (x400)×400) exposed to 4.266ppmNeem-On for 30 days showing damaging (D) and loosening of Hematopoietic tissue (LHT).



Discussion

There are great variations in the external structures of the kidney in fishes. The shape varies according to species. In teleosts, the kidney is distinguished into head and trunk kidney (Kumar & Tembhe, 2010). It serves as a main route of the excretion of metabolites and removal of xenobiotics. Histological alteration in the glomerulus of the kidney and in the tubular epithelium of fishes due to pesticides have been reported by many workers (Yokote, 1982; Kakuta *et al.*, 1997 and Mukherjee, 2000). They also suggested pesticides (hexachlorocyclohexan) produces necrosis/karyohexis and karyolysis in the renal tubules of *Labeo rohita*. Tilak *et al.* (2001) confirmed severe necrosis, cloudy swelling in the renal tubules and also reported cellular hypertrophy, granules in the cytoplasm and vacuolization in the cells of kidney of *Ctenopharyngodon idella* after exposure with fenvalerate. The acute and sub-acute toxicity of trichlorfon-induces histopathological changes has been described in the kidney tissue of *Prochilodus lineatus* (Veiga *et al.*, 2002). Ortiz *et al.*, (2003) and Cengiz, (2006) described histopathological changes in the kidney in the freshwater fish, *Cyprinus carpio* due to acute exposure to deltamethrin. The shrinkage in renal corpuscles clearly indicates that treated fish adopt some other routes of nitrogen excretion while the dilation of the renal corpuscles may be due to an increase in the filtration rate and consequently in urine volume, which may be a mechanism used by fish to overcome the toxic effect of the pesticide ((Thophon *et al.*, 2003., Roy and Bhattacharya, 2006, Velmurugan *et al.*, 2007). They also noticed necrosis of tubular epithelium, hypertrophied epithelial cells of renal tubules, narrowing of the tubular lumen, and expansion of space inside the Bowman's capsules and contraction of the glomerulus in the kidney of *C. mrigala*. Camargo *et al.* (2007) found cloudy swelling degeneration in the epithelium of renal tubules in the kidney of *P. lineatus* caged in Cambe stream, Brazil, polluted by industrial, domestic and agricultural wastes. These results are also in agreement with the changes in the kidney of Zebra fish (*Danio rerio*), exposed to sub-lethal concentration of chlorpyrifos (Scheil *et al.*, 2009) and on freshwater fish, *Piaractus mesopotamicus* exposed to organophosphate insecticide (Mataqueiro *et al.*, 2009 and Heo & Shin, 2009). Velmurugan *et al.* (2007) and Gill *et al.* (1989) reported pyknotic nuclei in the tubular epithelium, hypertrophied epithelial cells of renal tubules of *Cirrhinus mrigala* exposed to Monocrotophos. Gill *et al.* (1989) reported various histopathological changes such as degeneration of tubular epithelium, nuclear deterioration like karyohexis and karyolysis, and collapsing of glomerulus in the kidney of *Pontius conchoniuis* following exposure to cadmium. They also found progressive increase in severity of degenerative changes with increasing duration of exposure. The pathological alterations in the kidney of the studied fish are in agreement with these observed above by many investigators about the effects of different toxicants on fish kidney. Heo *et al.*

(2009) reported acute and subacute toxicity of trichlorfon in guppies (*Poecilia reticulata*). Vacuolated degeneration and necrosis of renal tubular cells was observed. Prashanth (2011) reported degeneration cells were frequently seen extruding into the Lumina of tubules, which were filled with fragments of cellular component. The pathological alterations in the kidney of the studied fish are in agreement with these observed above by many investigators about the effects of different toxicants on fish kidney. The present investigation confirms the findings of earlier investigation that trunk kidney of *Labeo rohita* shows histopathological changes due to pesticides

Conclusion

During the present study, it was found that so-called after environmentally friendly neem based pesticide Neem-On is toxic to *Labeo rohita* and also histopathological examination of the kidney of *Labeo rohita* clearly shows that normal architecture was lost.

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